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REVIEW OF TOXICITY TEST RESULTS SUBMITTED IN SUPPORT OF PESTICIDE TOLERANCE PETITIONS

by

Melvin D. Reuber, M.D.

for

U.S. Environmental Protection Agency
Office of Pesticide Programs

This report has been reviewed by the Office of Pesticide Programs and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Environmental Protection Agency.

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16. Abstracts Dr. Reuber examined a small selection of the thousands of pesticide toxicity test reports in EPA's files. He examined reports on chronic feeding studies in rats. In such studies, rats are fed diets containing a pesticide for extended periods (two years in most of the studies covered by Reuber). Such studies, together with several others required for registration and tolerance-setting, are designed to provide some indication of the health risks that may be associated with human exposure to pesticides. Test reports on 23 pesticide active ingredients were examined. These were selected from among the 275 active ingredients for which there are pesticide residue tolerances applicable to raw agricultural commodities. The ones selected are among those for which tolerances have been established for particularly large numbers of food commodities. He did not perform a detailed or definitive evaluation of the safety of each of the 23 pesticides. Nor did he perform a definitie evaluation of each test report, His charge was to make a general qualitative assessment of the adequacy of the test reports from the perspective of a knowledgeable and experienced scientist.

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FPA STATEMENT REGARDING DR. REUBER'S REPORT

Over the past several weeks, Dr. Melvin D. Reuber, an independent pathologist and EPA consultant, has been examining a small selection of the thousands of pesticide toxicity test reports in FPA's files. Such reports on testing with laboratory animals are submitted in support of pesticide registration applications and petitions for establishment of tolerances, i. e., maximum permissible limits, for pesticide residues in or on raw agricultural commodities. An interim report on Dr. Reuber's review is attached. Appended to it is information regarding his professional qualifications and experience.

Dr. Reuber examined reports on chronic feeding studies in rats. In such studies, rats are fed diets containing a pesticide for extended periods (two years in most of the studies covered by Dr. Reuber). Such studies, together with several others required for registration and tolerance-setting, are designed to provide some indication of the health risks that may be associated with human exposure to pesticides.

Test reports on 23 pesticide active ingredients were examined. These were selected from among the 275 active ingredients for which there are pesticide residue tolerances applicable to raw agricultural commodities. The ones selected are among those for which tolerances have been established for particularly large numbers of food commodities. As an unintended consequence, all the tests selected were performed between 1950 and 1970. This is due to the fact that, in general, the longer a pesticide has been registered, the more tolerances are likely to have been established. EPA will be examining

more recent reports in an auditing program it expects to start in the near future; until this is done, no definitive statement can be made on the extent to which more recent reports suffer from the same deficiencies that Dr. Reuber found.

Test reports such as those Dr. Reuber examined are reviewed by EPA scientists when the reports are first submitted; often, they are examined again when they are cited in support of subsequent registration applications or tolerance petitions. EPA reviewers may request explanations or clarifications, ask for additional data, suggest additional tests, or recommend that registrations be issued or tolerances set on the basis of the submitted reports. Dr. Reuber was not asked to examine the reviewers' comments. Neither did he have an opportunity to ask questions of the testing laboratories. His examination was limited to the reports originally submitted.

Examination of the reviewers' comments on some of the same reports has indicated that they frequently included criticisms similar to those made by Dr. Reuber. The following examples of reviewers' criticisms were culled from EPA files:

-- Low survival rate of experimental animals.

-- Lack of data on statistical significance of observed differences between experimental and control animals.

-- Inadequate histopatholical examinations; failure to report findings on all tissues studies; failure to report on examination of tumors in control animals; describing tumors as benign without histopathological examination.

Such deficiences commonly are the basis for EPA requests that petitioners provide additional information and/or clarification.

Dr. Reuber was not asked to perform, and he did not perform, a detailed or definitive evaluation of the safety of each of the 23 pesticides. Neither did he perform a definitive evaluation of each test report. His charge was simply to make a general qualitative assessment of the adequacy of the test reports from the perspective of a knowledgeable and experienced scientist.

Dr. Reuber's report reflects his own extensive experience as a pathologist specializing in cancer research. Thus, in providing his assessment of the test reports he reviewed, he has made detailed comments on their adequacy for purposes of making judgements on the tumor-inducing potential of the pesticides. His report suggests that the National Cancer Institute's recently published "Guidelines for Carcinogen Bioassay in Rats" may be used in some respects in planning and performing chronic feeding studies. EPA certainly agrees that testing of the tumor-inducing potential of pesticides is essential; indeed, such testing is required for pesticide registration and tolerance-setting. It is important to recognize, however, that many other factors must be taken into account in planning, performing, and evaluating toxicity testing. For example, EPA scientists believe that data on animals' blood chemistry and behavioral patterns are useful in making a comprehensive assessment of chronic toxicity; in such respects, EPA scientists are not in agreement with

Dr. Reuber's comments. In essence, while EPA scientists share many of the concerns expressed by Dr. Reuber, they have recommended that the reports he reviewed also be examined by scientists trained and experienced in other biomedical disciplines. EPA will undertake to have this more comprehensive review performed.

Dr. Reuber's findings do not necessarily mean that any of the 23 pesticides is dangerous to human health. Further investigation will be necessary before EPA can make a conclusive determination on this issue. In accordance with the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), all pesticide products previously registered must be reregistered by October 21, 1977. Each pesticide product will be reviewed, and a determination made as to whether or not to register it for particular uses, and whether each use should be classified as general or restricted. This process is being conducted in accordance with EPA regulations issued July 3, 1975 (40FR28242). Of the 23 pesticides involved in Dr. Reuber's review, 17 have already been identified as requiring additional chronic toxicity testing before they can be considered for full reregistration. See 41FR7218, dated February 17, 1976. Only one of the 23 was identified as a candidate for full reregistration; review of the other five has not been completed.

In addition, EPA is planning to start an auditing program, which will involve examinations of the laboratory records related to many of the test reports that Dr. Reuber reviewed. EPA will also audit laboratory records related to many other toxicity test reports already submitted, as well as many new reports, including reports submitted for purposes of

reregistration. Depending on audit results, EPA action will be taken in accordance with the existing regulations regarding pesticide registration, reregistration, and classification.

REVIEW OF TOXICITY TEST RESULTS SUBMITTED IN SUPPORT OF PESTICIDE TOLERANCE PETITIONS

BY

MELVIN D. REUBER, M.D.

Prior to registering pesticides or establishing tolerances for pesticide residues, the Environmental Protection Agency (EPA) requires that data be submitted regarding potential hazards to human health. Prospective registrants test pesticides in their own laboratories or in commercial or university laboratories.

I was asked by Office of Pesticide Programs to do a preliminary review of a series of reports submitted to EPA by various chemical companies which involved chronic animal exposure to certain chemical pesticides. On the basis of that review it has been recommended, and I concur, that a group of scientists carry out a more extensive, in-depth study as a follow up to my review. This report is based on a preliminary review of the data and includes my observations and conclusions from a biological medical viewpoint.

The reports of chronic rat studies for 23 pesticides were given to me for review.[1/] This report of my evaluation of these studies follows the following format: (1) plan of the experiment; (2) pathology protocol; (3) analysis of pathology and (4) statistical analysis; and finally, (5) remarks and conclusions that could be made from the studies.

1/ Even though these reports were designated as chronic studies, they also were generally regarded by the laboratories as oncology studies.

Because of the severe time restrictions placed on my effort, it was not possible to request further information from the chemical companies about their research laboratories, nor to write an in-depth review of each chemical, nor to analyze raw data in those instances when they were included. I was not made aware of any criticisms or conclusions of the reports which may have been made by EPA staff, nor was I aware of any requests for additional studies which EPA staff or officials may have made.

1. Plan of the Experiment

Charles River and Carworth Farm albino rats were most often used in the chronic rat studies; although sometimes the general term "albino" was given rather than the specific strain. Choice of strain is very important, and in fact one report stated: "The rats used . . . were of the type that were prone to develop tumors." Results are often more difficult to interpret when the controls develop a high rate of spontaneous tumors and/or other lesions.

All experiments used both males and females. The number of rats per sex and per dose level varied from 10 to 35, with one study using 50. Most often the number was 25, 30, or 35. Fifty rats of each sex per group are usually recommended for chronic feeding studies.[2/] Most studies did not mention how animals were chosen for the control and experimental groups, i.e., whether the selections were random. One study apparently replaced rats that died during the study with extra rats.

All studies except one for one year and another for 22 months were of two years duration. However, since in many studies part of the rats were killed after 26 and/or 52 weeks (with no indication given as to the criteria used to select the animals killed early), the number of

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2/ Sontag, J.N., Page, N.P. and Saffiotti, U.: Guidelines for Carcinogen Bioassay in Small Rodents. NCI Technical Report Series No.1.

rats at the end of the experiment was often smaller than at the beginning.[3/] The number of animals surviving until the termination of the experiment was also reduced in several of the studies because of excessive deaths due to pneumonia or other infectious diseases. In certain studies the cause of high mortality was not identified or stated.

Most studies included rather extensive hematology, urinalysis and limited blood chemistries taken at varying time intervals. These analyses provided little or no useful information; however,they could have been useful in cases of leukemia or bone marrow suppression.

Generally 3 different dose levels were administered. The rationale for choosing the doses used was never given although this may be documented elsewhere. Relevant background information concerning the chemical being tested was rarely included. Laboratories rarely confirmed the chemical used, the dose levels in the diets, or the stability of the chemical in the diet. Although in some cases laboratories may be testing compounds unknown to them, these factors nonetheless affect the reliability.

Although a few studies indicated that animals were "individually housed," most failed to include information concerning housing. Information was rarely given concerning how the animals at the various dose levels were identified. Housing of control rats and promimity to test rats is of great importance, to avoid inadvertent exchange of test and control rats and to reduce the exposure

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3/ In one study animals killed at 52 weeks had more histopathological lesions than those at the termination of the study.

of control rats to volatile test compounds. In some studies animals apparently were not observed frequently enough to allow autopsy of sick and moribund animals prior to development of advanced autolysis (decomposition of tissues that interferes with microscopic diagnoses).

In contrast to the general lack of detail concerning important matters such as housing conditions several studies presented in detail relatively unimportant clinical and behavioral observations of the rats during the course of the experiments. Since there were generally no differences observed between the control and test animals the question is raised as to the conditions, such as housing, under which the studies were carried out. Animals receiving high dosages of toxic chemicals would be expected to be more susceptible to minor diseases then normally healthy animals. The observations were no doubt included in order to contribute to an overall impression of thoroughness. Examples of these observations include the following:

"During the first year of the study, moderate respiratory involvement characterized by wheezing, nasal discharge, and rapid respiration was noted among the rats in all groups, including the controls. This is a common syndrome among laboratory rodents, and was unrelated to the feeding of . . ." (emphasis added)

"During the second year of the study, a gradually increasing number of animals in each group, including the control, had body sores, alopecia, and/or inflamed, protruding, or squinted eyes. 'Spinning' or circling to one side was noted in a few animals among the control and test groups. These signs are not uncommon among aged rats and are considered to be unrelated to the ingestion of.. . "(emphasis added)

2. Pathology Protocol

Complete gross autopsies were usually done; however, in very few studies were complete histologic examinations reported. Generally the most complete histology was reported only for the highest dose level test groups and the control groups, and then only at the termination of the study and based primarily on gross observation. Histology on lower dose level test rats and on rats killed after 26 and/or 52 weeks was extremely limited and sometimes completely absent. This is not satisfactory, since it is known that in many instances competing acute and even chronic toxicity at the highest dose levels may interfere with the health of the animals in such a way that fewer lesions may develop in high dose animals than in animals fed at the lower or intermediate dose levels. Furthermore, microscopic tumors of organs, particularly of endocrine organs,will be missed completely because they were not observed or recognized on gross examination.

Tumors in several studies, particularly subcutaneous tumors and pituitary tumors, were not examined histologically, because they were considered to be "spontaneous" and of no importance. Not only is it necessary to section tumors histologically, but often multiple sections of large masses are needed to make the correct diagnoses. The subcutaneous tumors often were large and no doubt the cause of death in some or all of those animals in which they occurred. In at least one study these subcutaneous tumors were autolyzed and the diagnosis was

difficult, if not impossible to make. All subcutaneous tumors are not merely fibroadenomas or fibromas of the mammary gland which do often occur in control as well as in test animals, but may be adenocarcinomas or fibrosarcomas, leiomyosarcomas, rhabdomyosarcomas or reticulum cell sarcomas. Pituitary tumors likewise may be carcinomas and are not invariably adenomas. In one study histologic diagnoses were not given for markedly enlarged spleens or for abnormal pituitary glands. It was pointed out in another study that:

"It is, of course, obvious that the actual incidence of mammary gland tumors is greater than would be apparent from gross observation only and that such tumors may not be detected in a routine microscopic examination unless multiple or serial sections are examined. It is also of particular interest that four of the six mammary gland tumors detected in the rats fed the two highest levels of . . . in the present studies appeared microscopically to be malignant."

Autolysis (decomposition) of tissues was a major problem since such animals were either not autopsied, incompletely autopsied or no tissues were saved.[4/] Most studies reported:

"Severe autolytic changes in the tissues of several animals. . . . prevented microscopic examination of all grossly evident tissue masses."

"If marked autolysis was noted, only the lung, liver, and kidney were examined."

"There are no significant alterations, aside from frequent congestion and autolysis in the section of kidney, liver and spleen."

4/ The histologic sections of a mouse study done by FDA were given to one company for pathological evaluation. An unspecified number of animals in that study was excluded because of autolysis.

"There is no evident drug effect, although autolysis makes a critical parenchymal examination impossible in numerous cases."

"Autopsies were performed . . . from every . . . animal with the exception of those in which autolysis was advanced."

"Throughout this entire group the predominating findings seemed to be as follows: a marked autolysis,. . ."

Microscopic and gross observations were usually not correlated. The gross lesion was ignored or assumed to be inaccurate if a histologic section did not confirm such a lesion. The same person probably does not write the gross description and examine the histopathology. Gross examination and description most likely were done by technicians and the histopathology by pathologists, without additional correlation of the two.

Failure to correlate the gross with the histopathology can result in the misdiagnosis or lack of diagnosis of tumors. "An apparent hemorrhage in the muscle of the hip" was not diagnosed histologically and could have been a hemangioendothelial sarcoma. Bloody areas, cysts, or apparent hemorrhages in other organs such as liver or lung, likewise can be sarcomas. Another study described "among the females of each group that of enlargement of hemorrhagic appearance of the pituitary" without corresponding microscopic diagnoses. Records might indicate further instances of descriptions of gross lesions without histologic study.

Body weights, selected organ weights and sometimes food consumption were often recorded and analyzed statistically. This data was apparently included in order to support the conclusions that there were no

statistically significant differences between control and test animals. However, when there were significant differences, they usually were not incorporated into the discussion of the results or the conclusions. In a few instances in which "statistical outliers" (organ weights or chemistry values that were out of line with the remaining values) were excluded, this resulted in a finding of no differences between the control and test rats.

The terminology for diagnosis varied considerably from one laboratory to another. In addition, some diagnoses need to be clarified. For example, large subcutaneous masses responsible for death were diagnosed as "cystic fibrosis" or "adenosis" rather than as tumors. In another instance a very large mass was diagnosed as "organizing granulation tissue." The diagnoses for some spleen were "lymphocytic tumor"; however, the greatly elevated white blood counts and the markedly increased splenic weights strongly suggest leukemia. Such diagnoses as metastasizing fibroadenoma "involving the left kidney and surrounding structures" and cellular fibroma "involving urinary bladder and ancillary tissues," were used even though invasion and metastases are considered to be signs of malignancy. The wide variety of terms used to diagnose lesions of the liver was illustrated by "hyperdysplastic, hypodysplastic and hypertrophic" nodules.

Often lesions were described without efforts at explanation. Other changes were explained away with poor or inappropriate reasoning. Examples include (emphases added):

"The swollen pale, "blown-up" cells probably represent hydropic change and this is possibly due to prolonged congestion. In addition, since the changes are seen with Bouin's fixative material more than with formalin it is felt that there is some element of fixative artifact. In any case. the swollen cells certainly represent change which is reversible." (Hydropic change was seen in tissues fixed in either fixative. There is no documentation that this change is the result of congestion or is, in fact, reversible.)

"Occasional lenticular degenerative change was recorded but no significance can be attached to this observation because of the many artifacts that appear during routine processing of this organ." (Some effort should have been made to use more suitable methods of processing.)

"The atypical alteration in the epithelium of the urinary bladder in animals . . . may reflect a toxic effect. . . This type of change appears to be of a metaplastic rather than of a neoplastic nature." (Is this change in the urinary bladder a prenoeplastic lesion that would have become a tumor had the animals lived longer?)

"Interpretation of this information is difficult, because of the wide variation between animals in each group, and because the changes involve hematopoeitic tissue, which cannot be accurately studied using routine histologic techniques." (Routine histologic techniques may be adequate; however, if not, why not use suitable ones?)

"All of them, however, are of a benign nature and are not tumorous in the sense of the word but are tumor masses." (referrring to tumors of the mammary gland)

3. Analysis of Pathology

There was inadequate presentation, tabulation and analysis of the data, and often there were insufficient data included to make an accurate analysis possible.

The data were frequently presented in poorly designed tables and in an incomplete manner; it was difficult or impossible in many instances to ascertain total number of tumors versus total number of animals with tumors in each group. Total numbers of animals at risk (numbers necessary for proper analysis of data) were not always given. Tabulation of lesions was often limited to tumors only, and not other lesions, and often to "internal tumors" with the exclusion of subcutaneous or skin tumors. Such data can be obtained only by requesting and reviewing the raw data in greater detail.

Data presented in several tables were misleading or distorted. Several studies included "palpable tissue masses, nodules or wart like lesions in the skin" in the same category. The wart like lesions weere not examined histologically. Tumors occurring in both control and test animals were listed, whereas uncommon tumors that occurred in only test animals were omitted from the tabular data.

Confirmation of the summary data by examination of the raw data was not easy. Even without extensive examination, it was apparent in a few cases that tumors had been included in the raw data and yet were omitted from the tables. It was not possible to

ascertain if clerical or arithmetic errors resulted in reports of fewer tumors. It appears that little effort has been made by the laboratories to verify information in the tables.

Benign versus malignant tumors, separation of tumors by dose levels, correlation of lesions by sexes, single organ site verus multiple organ involvement, and discussion of "unusual or rare" tumors rarely or never seen in control animals of the same species and strain, and total number of tumors are all important. The data were usually not presented or analyzed in categories reflecting the above important distinctions.

Benign tumors often occur in the controls, particularly in the mammary gland or pituitary in females. Consideration should be given to earlier tumor appearance, earlier animal death, increase in tumor incidence, and multiple tumors at single sites when comparing such tumors in test and control animals. Mammary gland tumors and pituitary tumors may also be malignant in test animals and benign in the controls. In some organs benign tumors may be a stage in the development of malignant tumors and this should be taken into consideration.

Discussions of tumor incidence usually did not distinguish between sexes. This is significant because, for example, mammary gland tumors and pituitary tumors generally occur more often in females than in males while male rats are more susceptible to the development of tumors of the liver. Thus, for a thorough and meaningful analysis it is important to provide a complete break down by sex.

The reports did not group tumors according to the organs in which they were found. Further, the discovery of "unusual or rare" tumors was not discussed. Tumors of the kidney, rarely seen in controls, may be significant even in small numbers in test animals. Hemangioendothelial sarcomas of the liver have not been reported in the literature in control rats not treated by chemicals. If such rare tumors occur in the controls, some evidence must be given to rule out the possibility that experimental and control animals were mixed.

Some rare or unusual lesions or tumors observed in test animals in the studies, but not noted as such by the authors, include hemangiosarcoma of the kidney, reticulum sell sarcoma of the brain, nephroblastoma, carcinomas of the pancreas, highly analplastic carcinoma of the kidney with metastases to several other organs, adenocarcinoma of the stomach, carcinoma of the prostate mesothelioma of the pleura, and hepatic vein thromboses sometimes with infarcts of the liver.[5/]

Tabulation and analysis of lesions other than tumors, particularly necrosis of liver and kidney and chronic renal disease, were often omitted.[6/] Necrosis was difficult to observe in dead rats because

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5/ Rare or unusual highly malignant tumors, including carcinoma of the prostate were found in rats ingesting heptachlor epoxide. Reuber, M.D.: Statement for testimony at Heptachlor/Chlordane hearings.

6/ Rats ingesting Mirex developed a statistically significant incidence of renal disease Reuber M.D.: Statement for testimony at Mirex hearings.

"Part of this finding may have been due to an early incidence of a disease syndrome which involved the lungs, liver and heart. Certainly, the compound added its effect to this already stressed condition." (increased mortality)

"We must, however, qualify this statement by drawing attention to the fact that the general condition of the animals was poor on account of the chronic infections in the cages." (the study was reported as negative for carcinogenicity)

". . . life was maintained nearly entirely by the hypodysplastic nodules (. . .) since the small fraction of parenchyma left was severely altered by toxic reaction and compression by the nodules. . ." (liver)

"Again, this is probably better referred to as a desmoplastic mass of connective tissue producing atrophy of the mammary gland." (mammary gland tumors)

"Externally palpable tissue masses, for the most part associated with mammary glands, are a common finding in aging laboratory rats."

"This is a common finding in the urine of aged albino rats." (proteinuria)

"In addition to these rather subtle changes many of the animals possessed abscesses that were observed grossly. . . and were primarily located at the hilus of the liver." (Abscesses of the liver are a rare finding)

"Histological findings do not indicate permanent tissue damage produced by . . ., with the possible exception of changes in the testes. Interpretation of these results is somewhat complicated, inasmuch as the changes are equivocal and . . ."

"Organ weight findings suggest increase in size of liver and kidney, but these changes do not appear to be significant inasmuch as the weights of control organs were unusually low and the weights of the organs obtained from the experimental animals are essentially within normal limits."

advanced autolysis had been allowed to occur. Most often chronic renal disease was ignored. In one study it was described in great detail and felt to be significant, but in other studies from the same laboratory this lesion was given little attention. Lesions of the testes, which were also observed in a few studies, were unexplained.

It is not always necessary to have a dose response with regard to the development of tumors. Toxicity, particularly at higher dose levels, interferes with the development of tumors by causing early death, poor health, or development of other chronic diseases. Furthermore, the target organ may be the liver at the higher doses, but shift to the lungs or other organs at the lower dose levels.[7/] Conclusions such as "the lack of a positive dose-related increase in all tumors or tumors of any one tissue demonstrate the absence of a carcinogenic effect attributable to continuous exposure to . . ." are not justified.

Discussions and summaries which attempt to belittle the significance of such effects in test animals can be seen in the following statements (emphases added):

7/ Gross, A.: Statement for testimony at Dieldrin/Aldrin hearings.

"The micropathological findings in the organs or tissues of the test animals including the relatively small, widely scattered incidence of tumorous masses are apparently of coincidental nature and unrelated to the ingestion of . . . ."

"In spite of the large amount of abnormal findings, which were more or less consistent throughout these animals, there was no evidence in any of the tissues that were in a better state of preservation which showed any change that could be associated with the experimental material either in the cells or in the tissues. It is therefore believed that these animals died from the usual changes associated with aging and not as the result of any experimental material. The control and experimental animals showed similar changes."

"Some thyroid sections from both the control and test groups showed. . .; these deviations were considered to be of no significance."

"One early death was due to 'diseased kidneys' in one female rat . . . cannot be attributed to the material under study."

"No significant histopathologic changes were observed in any of the test rats that could be attributed to the feeding of . . . All changes observed were considered to be the effects of spontaneous disease in rats."

"It is possible but not likely that these were produced by . . ."(thyroid lesions).

"As seen in the table above, the increased or decreased organ weight data for the test groups did not follow a consistent pattern and are not meaningful."

"Spontaneous microscopic alterations were seen in nearly all of the organs examined from the animals on this study, but neither their incidence or severity was greater in the test animals than in the controls."

"Microscopic examination of tissues from the test rats sacrificed at termination of the study did not reveal any histopathological alterations that could be attributed to the ingestion of the test material. Degenerative or neoplastic lesions encountered in control and test rats were compatible with those commonly occurring in rats of this strain and age."

4. Statistical Analysis

Statistical analysis of the pathological findings is, in all but one study, absent. This omission, in view of the poor presentation, tabulation and analysis of the raw data, is hardly surprising. In one study in which there were multiple tumors, statistics were used to show that the study was negative. However, the original data were reviewed by another independent investigator, in addition to myself (an extremely time consuming review) and we both concluded that there was evidence to suggest an increase in tumors caused by the chemical. 8/

The statistical analysis of the pathological findings should include not only the total incidence of tumors, but also the incidence of tumors in the other categories discussed under Analysis of Pathology (see page 12) as well.

One important factor in statistical analysis is the number of animals in any one test or control group considered to be at risk. In many studies, animals that die early in the study from infectious diseases have not been on the test compounds long enough to develop tumors or other lesions. These animals should not be included in the final results.

The only way to analyze numbers of animals in any one study is to include only those animals surviving for a period of time equal to, or longer than, the time of appearance of the first tumor as being

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8/ The independent corroboration of that study was done by Sidney M. Wolfe of the Health Research Group.

at risk for that tumor. This factor has been ignored in some studies which thereby give misleading low percentages of tumors because the experimental animals tend to die earlier than the controls.

No pattern was apparent in the studies as to which animals were included in the results. Some included all animals; others included those that lived longer than one year. In one study which included only two year survirors, some of the test groups had only three surviving rats, but some animals in all groups, including controls, had tumors. The conclusions that there were no differences between the control and test animals were not warranted. The histopathological data on the rats that did not survive two years either was not available or was not made available.

It is necessary to distinguish and separate hyperplastic nodules from carcinomas of the liver. One the one hand the incidence of carcinomas of the liver will be decreased if carcinomas are included with nodules. On the other, the number of carcinomas will be increased if hyperplastic nodules are counted as carcinomas. The overall effect can be misleading in several ways. In the first instance, the incidence of malignant tumors would be lowered in experimental animals. In the second instance, the incidence of tumors of the liver in controls animals would be increased. The overall effect would make a chemical appear less carcinogenic for the liver. 9/

9/ This is particularly true in studies using mice that may develop nodules of the liver spontaneously, whereas the test mice develop carcinomas Reuber, M.D.: Statement for testimony for hearings on Dieldrin/Aldrin

Some studies included animals that had advanced autolysis (decomposition of tissues) and therefore no histologic evaluation. One study stated:

> "Strictly speaking, the analysis should have been based on only those animals receiving a full post-mortem. However, since the groups received equitable treatment in this respect, using all deaths within a period. . ." and "The results showed a somewhat higher incidence of tumors in animals that underwent complete necropsy."

There is no valid reason for the exclusion of statistical outliers (very large values that were out of line with the remaining values) from the data. In two studies:

> "In several instances single organ weights or organ-to-body weight ratios did not conform to the other values in the groups in which they occurred, thus causing the means of the groups to be widely distorted; therefore, these values could be justifiably deleted from the data."
>
> ". . . and in these summaries some of the means shown in the detailed tables have been recalculated after excluding one or two very large values."

Even though the results of the pathology studies were not analyzed statistically, there was excessive analysis of other data.

> "The criteria chosen for statistical analysis were survival at 26, 52, and 104 weeks; body weight gains from 0-52 weeks; total food consumption from 0-13 weeks, organ weights and organ-to-body weight ratios for those animals sacrificed at 26 and 104 weeks."

While I am not a statistician, my own experience with biological and pathological aspects of medical research leads me to believe that a qualified statistician should review the studies and confirm the presence or absence of significant lesions.

5. Remarks and Conclusions

Bias in the plan of an experiment, carrying out of an experiment, or in the analysis of a study, in a lot of innocent or minor appearing ways, can influence the final results and conclusions of the study. Some of these biases which may enter into these studies include the following:

- failure to randomize animals for the various groups;
- choosing animals with lesions to be killed after 26 and/or 52 weeks;
- failure to section particular lesions histologically;
- including autolyzed animals in the tissues examined but failing to perform histopathology;
- using terminal animals for bio-chemical and other studies, but failing to include autopsy or histology examination;
- killing an animal with a skin tumor or mammary gland tumor early, or excising, benign mammary gland tumors so that they may not become malignant later in the experiment;
- failure to end a study at a point where the test animals have more tumors than the controls, or failure to extend the duration of studies to a point where the test animals may have a chance to develop more tumors than the controls;
- choosing a strain of rat that has a high incidence of spontaneous tumors;
- choosing pathologists with insufficient or inappropriate experience; 10/

10/ Recommended decision of Chief Administrative Law Judge, Velsicol Chemical Corporation FIFRA Docket No. 384, pp. 83-85.

Most of the studies were reported as essentially negative with little or no changes that could be attributed to the compound tested. Only one study could be readily determined as satisfactory. In many of the studies no conclusions could be made from the study as reported; however, it may or may not be possible to decide if these particular studies are negative or positive if further extremely time-consuming analyses of the data are done. Indeed one study reported as negative was probably positive for carcinogenicity. In several other cases there was insufficient data to analyze, so that additional data is needed before conclusions can be reached.

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